PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	17.05.2004
Applicant's or agent's file reference: 03.0580-B	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/ES 03/00140	25.03.2003	26.03.2002

Applicant: ALEMANY BONASTRE, RAMON et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
03.0580-B		

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/ES 03/00140	25.03.2003	26.03.2002

International Patent Classification (IPC) or both national classification and IPC
A61K35/76

Applicant
ALEMANY BONASTRE, RAMON et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23.10.2003	17.05.2004

Name and mailing address of the international preliminary examining authority:	Authorized Officer
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/ES 03/00140

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-7, 10-24	as originally filed
8a, 9a, 10a	received on 22.10.2003 with letter of 22.10.2003
24b, 24c, 24d	received on 15.04.2004 with letter of 13.04.2004

Claims, Numbers

1-7	received on 22.10.2003 with letter of 22.10.2003
8-11	received on 15.04.2004 with letter of 13.04.2004

Drawings, Sheets

1/9-9/9* received on 15.04.2004 with letter of 13.04.2004
See Separate Sheet

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:
   - ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item I

Basis of the report

1- The amendments filed with the letter dated 13.04.04 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are pages 24b-24d (examples 4 and 5) and the corresponding figures 7-9.

New claims 8-11 meet the requirements of Article 34(2)(b) PCT.

1.1- Hence, for the purpose of this report, the present application has been considered as if pages 24b-24d (examples 4 and 5) and the corresponding figures 7-9 had not been added to the application.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2- Reference is made to the following documents:

D1: WO-A1-0135970
D2: WO-A2-9835028
D3: US-A-5002874

The relevant passages are those indicated in the search report, unless otherwise specified.

NOVELTY - Art. 33 (1) and (2) PCT

3- Claims 1-11 can be considered as being formally novel over the prior art documents cited in the search report.

3.1- D1 claims the use of modified adenovirus for the treatment of a Ras-mediated cell proliferative disorder in a mammal having a Ras-activated pathway (see claim 1), in which the modified adenovirus lacks the gene encoding VAI RNA (see claim 2 and examples).

Present independent claim 1 is however directed to the use of an adenovirus defective in its VAI **and** VAII virus-associated RNAs. Hence, D1 does not appear to be novelty destroying for the subject matter of present claims 1-11.

3.2- D2 relates to a complementary-Ad vector system for killing tumor cells and mentions E1 deletion or substitution by the replacement of the E1a promoter by one which is active mainly in hepatocarcinoma cells or by deletion or mutation of E1b-p55 or E1b (see also the claims).

Hence, D2 does not appear to be novelty destroying for the subject matter of present claims 1-11.

INVENTIVE STEP - Art. 33 (1) and (3) PCT

4- Claims 1-11 can be considered as being inventive over the available prior art:

4.1- The closest prior art is represented by D1 (see item 3.1- above).
The closest prior art differs from the present application in that it does not mention a further defect / mutation in VAII.
The technical effect achieved in the present application is the provision of a better (more selective) cancer treatment.
The objective problem posed in the present application is to provide further adenovirus mutants for the purpose of treating cancer.
The solution proposed is an adenovirus defective in its VAI and VAII RNAs.

4.2- Technical evidence has been provided showing that an adenovirus with both VAI and VAII RNAs mutated is more selective for tumor cells with an active Ras pathway than an adenovirus with only the VAI RNA mutated.

Furthermore, it is nowhere suggested in D1 alone, or taken in combination with D2 to provide an adenovirus defective in its VAI and VAII virus-associated RNAs.

Hence, claims 1-11 can be considered as being inventive over the available prior art.

INDUSTRIAL APPLICABILITY - Art. 33 (1) and (4) PCT

5- Claims 1-11 can be considered as being industrially applicable.

Example 4. Construction of an adenovirus mutated in VAI and VAII RNAs.

To delete both VAI and VAII from the adenovirus genome, restriction enzymes Xba I and BspE I were used applying standard molecular biology techniques. Xba I (sequence TCTAGA) cuts 4 times along the adenovirus type 5 genome and one of those cuts is located 30 bp before the VAI RNA gene (see Figure 2). BspE I (sequence TCCGGA) cuts 9 times along the adenovirus type 5 genome and one of those cuts is located 20 bp before the end of the VAII RNA gene (see Figure 2). Therefore, these specific Xba I and BspE I sites flank the region from bp 10590 to 11011 of Ad5 that contains the VAI and VAII RNA genes. In order to cut only at these sites, a Sal I fragment of Ad5 was subcloned into a small plasmid (pCT) so these Xba I and BspE I sites become unique sites. After cutting with Xba I and BspE I, the deletion ends were filled with klenow polymerase and religated. This procedure resulted in a 418 bp deletion that removes VAI and VAII RNA genes (named "VAdel" deletion). The deletion point is flanked by the klenow-filled XbaI and BspE I sites: TCTAG-CCGGA. The deletion was transferred to the rest of the adenovirus genome in two steps: one homologous recombination and one ligation of an AscI fragment of the Ad5 genome (see Figure 7 for the complete strategy). The plasmid with the complete Ad5 genome except for the 10590 to 11011 deletion is named pAdVAdel. Virus AdVAdel was obtained from this plasmid by releasing the genome with restriction enzyme Pac I and transfecting it in 293T cells. This cell line was used

because it is known that SV40 T-antigen can complement defects of Adenovirus VA RNAs. After amplification and purification of virus "AdVAdel" the sequence form its genome shows the deletion of both VAI and VAII (Figure 7 lower pannel).

Example 5. An adenovirus with both VAI and VAII RNAs mutated is more selective for tumor cells with an active Ras pathway than an adenovirus with only the VAI RNA mutated.

To measure the selectivity of the viruses described in the invention, we compared the IC50 (concentration of virus in viral particles per cell that produces 50% of cell lysis) in cells with an inactive (293 cells) or an active Ras pathway (NP9 cells). The adenoviruses tested were Adwt (no deletions), dl331 (VAI deletion) and AdVAdel (VAI and VAII deletions). The methods are the same as described in Example 2. The results are presented in Figure 8. In 293 cells, AdVAdel was more defective than dl331 and Adwt. Compared to Adwt the defectiveness is in the order of 300-fold. Compared to dl331, AdVAdel is 10-fold more defective in cells with non-active Ras. This defectiveness achieved in cells with non-active Ras pathway was also demonstrated at the level of viral protein production. Structural proteins synthesized at a late phase of the virus replication cycle were detected by western blot using a polyclonal antibody. As shown at the upper right panel of Figure 8 the defectiveness compared to Adwt of the VAI and VAII deleted mutant is clearly observed and very superior to the defectiveness of the VAI mutant dl331. In contrast,

in NP9 pancreatic carcinoma cells with an active Ras pathway AdVAdel is not defective compared to Adwt and dl331 both at the level of cytolytic effects and late protein expression (Figure 8 lower panels).

To demonstrate that the adenovirus with mutated VAI and VAII shows Ras-dependent replication we used transient transfections with active (V12) or negative-dominant (N17) forms of Ras as presented in Example 1. The cytolytic effects of AdVAdel virus were compared to Adwt in each transfected cell population (Figure 9). In mock-transfected (GFP-plasmid control) 293 cells, AdVAdel was 320-fold more defective than Adwt. When the Ras-pathway was activated with Ras-V12 this defect was eliminated. Conversely, a further inhibition of the basal Ras-pathway in 293 cells led to an attenuation of AdVAdel of 3500 times compared to Adwt. This level of Ras-dependent replication measured using this transient transfection assay is much higher than the observed for the single VAI mutant dl331 (20 times as shown in Example 1).

8. Use according to any one of the preceding claims, wherein said adenovirus has mutations in the VA RNA genes that confer selective replication on tumor cells and that, in turn, contain other genes commonly used in the field of cancer gene therapy such as prodrug activators, tumor suppressors, or immunostimulants.

9. Use according to any one of the preceding claims, wherein said adenovirus is a human adenovirus derived from a serotype between 1 and 50 with genetic mutations in the VA RNAs genes that confer selective replication on tumor cells.

10. Use according to Claim ~~11~~ 9, wherein said adenovirus is a human adenovirus derived from serotype 5.

11. Use according to Claims ~~11~~ 9 to ~~12~~ 10, wherein said adenovirus is a mutant adenovirus dl331.

FIGURE 7

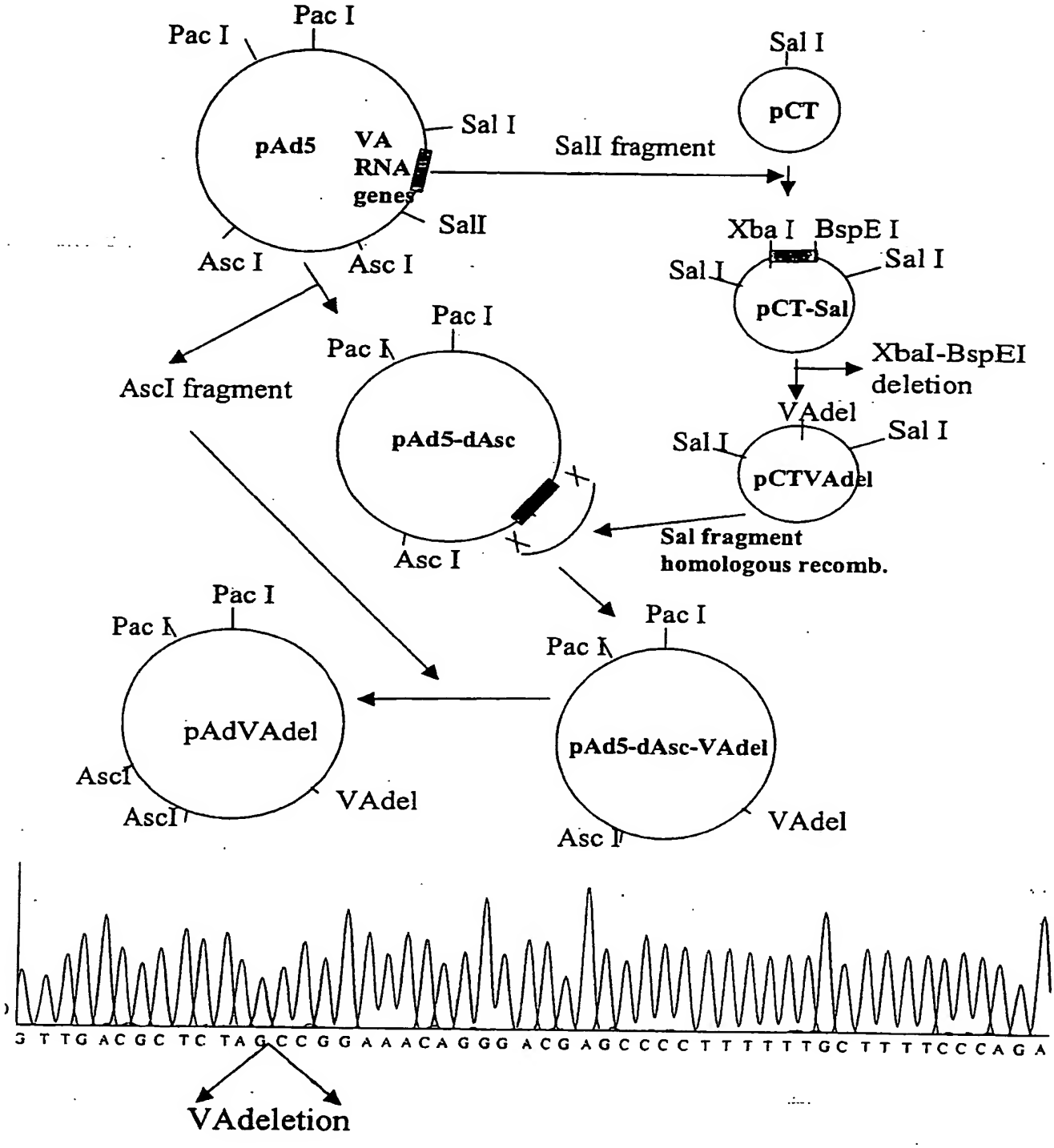
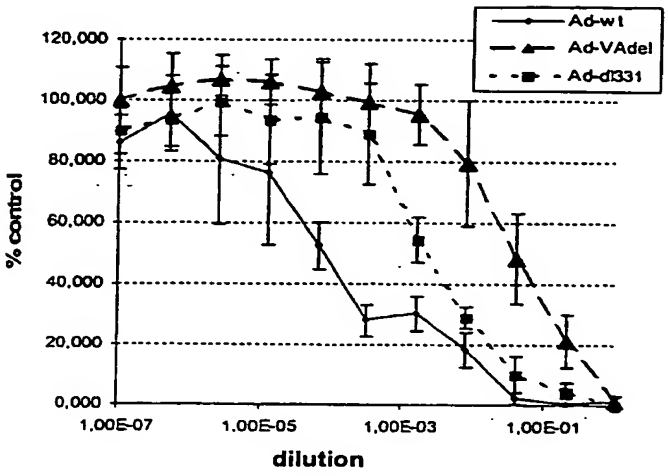
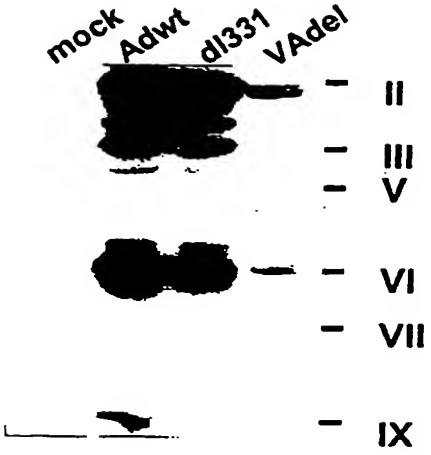

Pac I
Pac I
pAd5
VA RNA genes
Sal I
SalI
Asc I
Asc I
SalI fragment
Sal I
pCT
Xba I
BspE I
Sal I
Sal I
pCT-Sal
XbaI-BspEI deletion
VAdel
Sal I
Sal I
pCTVAdel
Sal fragment homologous recomb.
AscI fragment
Pac I
Pac I
pAd5-dAsc
Asc I
Pac I
Pac I
pAdVAdel
AscI
AscI
VAdel
Pac I
Pac I
pAd5-dAsc-VAdel
Asc I
VAdel
G T T G A C G C T C T A G C C G G A A A C A G G G A C G A G C C C C T T T T T T G C T T T T C C C A G A
VAdeletion

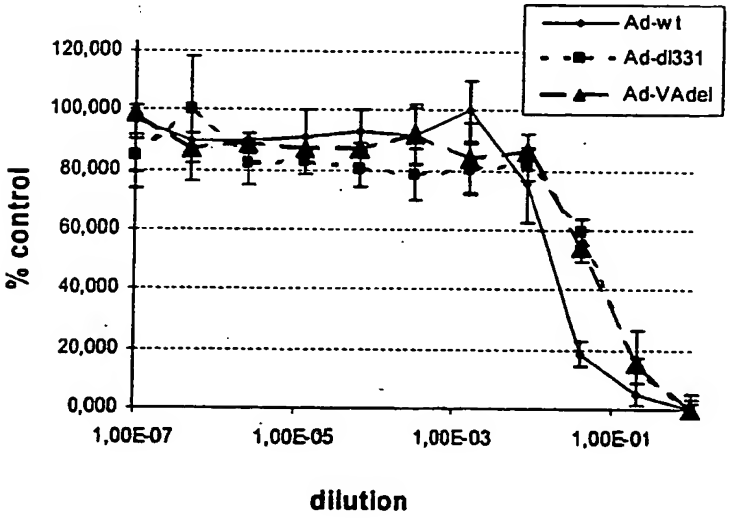
Figure 8

293 cells





NP9 cells



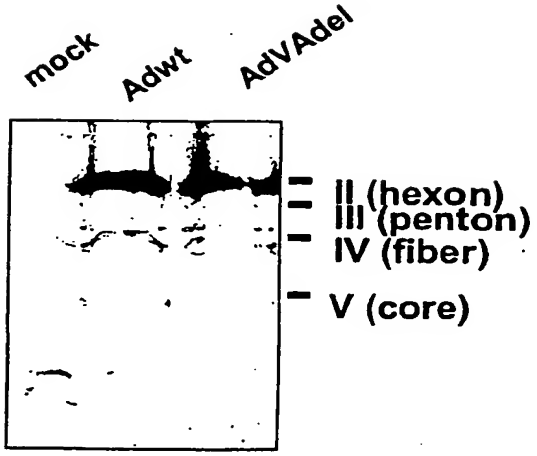
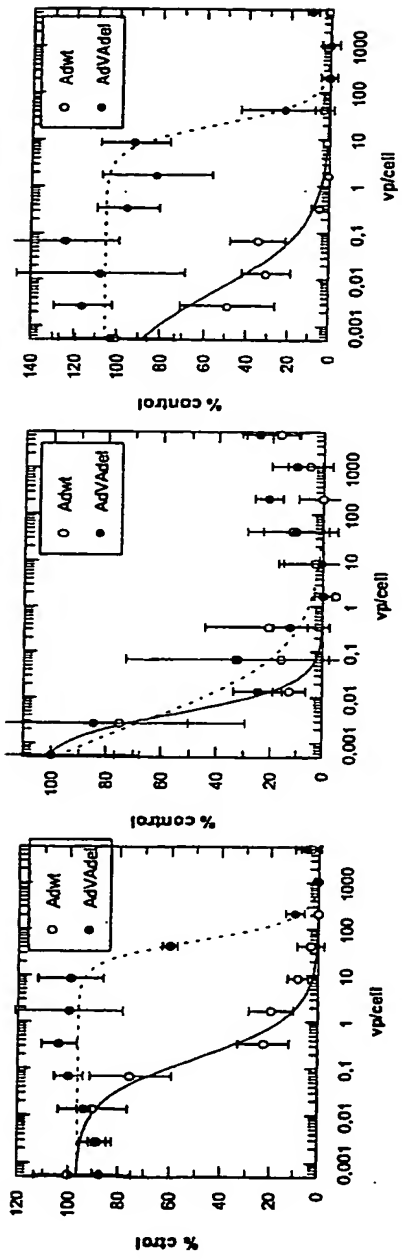


Figure 9



	mock	HrasV12	HrasN17
IC_{50} *(Ad-wt)*:	0,18 v.p./cell	0,005 v.p./cell	0,006 v.p./cell
IC_{50} *(Ad-VAdel)*:	57,9 v.p./cell	0,003 v.p./cell	21,0 v.p./cell
Ratio:	***320***	***0,6***	***3500***